IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Elliott Richelson et al. Art Unit: 1635
Serial No.: 08/953,269 Examiner: S. McGarry
Filed : October 17, 1997
Title : USING POLYAMIDE NUCLEIC ACID OLIGOMERS TO ENGENDER A BIOLOGICAL RESPONSE

Assistant Commissioner for Patents
Washington, DC 20231

DECLARATION UNDER 37 CFR §1.132 OF ELLIOTT RICHELSON

I, Elliott Richelson, declare as follows:

1. I am a citizen of the United States and presently live at 109 Teal Pointe Lane, Ponte Vedra Beach, FL 32082-1936.

2. I am presently employed by Mayo Foundation, and have been so employed since 1975.

3. I received a Doctor of Medicine Degree from Johns Hopkins University, School of Medicine, Baltimore, MD.

4. I am an inventor on the above-indicated patent application.

5. I signed a Declaration that was submitted to the Patent and Trademark Office on October 26, 2000.

6. While preparing a Declaration for a related case having serial number 09/168,714, a review of the notebooks from my laboratory revealed several inadvertent errors in Paragraph 12 of the Declaration submitted October 26, 2000.

7. Paragraph 9 below is a corrected version of Paragraph 12 of the Declaration submitted October 26, 2000. Thus, Paragraph 9 below should be read in place of Paragraph 12 of the Declaration submitted October 26, 2000.

8. Paragraph 10 below is a description of additional experiments related to the experiments described in Paragraph 9 below.

9. Individuals under my supervision conducted experiments using a PNA oligomer targeting the coding strand of rat β-amyloid precursor protein (sense β-APP-PNA) and either a saline control or a control PNA oligomer similar to the sense β-APP-PNA with the exception that it contained a mismatch at every third nucleotide position (mismatch β-APP-PNA). The levels of Aβ(1-40) and Aβ(1-42) protein were measured in three experiments. In one of the three experiments, the levels of Aβ(1-42) protein were measured in sense β-APP-PNA-treated animals and saline-treated controls with no statistically significant difference being detected. In the other two experiments, however, animals treated with the sense β-APP-PNA exhibited a significantly lower level of Aβ(1-42) protein when compared to the level exhibited for controls (the control for one experiment was saline-treated animals, and the controls for the other experiment were saline-treated animals and mismatch β-APP-PNA-treated animals). In all three experiments, no difference was detected between the levels of Aβ(1-40) protein measured for the sense β-APP-PNA-treated animals and control animals. It is noted that a PNA oligomer targeting the non-coding strand of rat β-amyloid precursor protein (antisense β-APP-PNA) was administered to animals. After administration, the levels of Aβ(1-40) and Aβ(1-42) protein were measured and compared to the levels measured in animals treated with saline. No statistically significant difference was detected. It also is noted that a second PNA oligomer targeting the coding strand of rat β-amyloid precursor protein (sense β-APP-PNA2) and a second PNA oligomer targeting the non-coding strand of rat β-amyloid precursor protein (antisense β-APP-PNA2) were administered to animals. After administration, the levels of Aβ(1-40) and Aβ(1-42) protein were measured and compared to the levels measured in animals treated with saline or a control PNA oligomer (mismatch β-APP-PNA2) similar to the sense β-APP-PNA2 with the

exception that it contained a mismatch at every third nucleotide position. No statistically significant difference was detected.

10. The following experiments were conducted in mice. The levels of mouse Aβ(1-40) and Aβ(1-42) protein were measured in blood and brain samples collected at different time points (4, 8, 12, 16, and 24 hours post-injection) from mice treated with a single intraperitoneal injection of the sense β-APP-PNA referenced above. These levels were compared to the levels measured in mice treated with saline or the mismatch β-APP-PNA referenced above. No statistically significant difference was detected between the sense β-APP-PNA-treated mice and control mice when the plasma levels of mouse Aβ(1-40) and Aβ(1-42) protein were measured. Mice treated with the sense β-APP-PNA did exhibit a significantly lower level of Aβ(1-40) and Aβ(1-42) protein in their brains at the 16 hour post-injection time point when compared to the brain levels exhibited for control mice. In addition, mice were treated with a single intraperitoneal injection containing one of the following nine PNA oligomers: three PNA oligomers targeting the coding strand of mouse β-amyloid precursor protein, one PNA oligomer targeting the non-coding strand of mouse β-amyloid precursor protein, three PNA oligomers targeting the coding strand of mouse beta-amyloid cleaving enzyme (BACE), and two PNA oligomers targeting the non-coding strand of mouse BACE. Control mice were treated with either the mismatch β-APP-PNA or saline. Seven of the PNA oligomers targeting either mouse β-amyloid precursor protein or mouse BACE that were administered to mice resulted in no difference in plasma Aβ(1-40) or Aβ(1-42) protein levels when compared to the levels measured in either the mismatch β-APP-PNA-treated mice or saline-treated mice. One sense PNA oligomer targeting mouse β-amyloid precursor protein (sense -47APP-PNA) resulted in a 21 percent decrease in plasma Aβ(1-40) protein levels ($p<0.15$), while one antisense PNA oligomer targeting mouse BACE (antisense +29BACE PNA) resulted in a 25 percent decrease in plasma Aβ(1-40) protein levels ($p<0.13$). In another experiment, mice were injected with four daily injections of either the sense β-APP-PNA, the sense -47APP-PNA, the antisense +29BACE PNA, or saline. The sense β-APP-PNA-treated mice and antisense +29BACE PNA-treated mice exhibited plasma Aβ(1-40) protein levels comparable to those measured in saline-treated mice. The sense -47APP-PNA-

treated mice, however, exhibited a 31 percent decrease in plasma Aβ(1-42) protein levels when compared to saline-treated mice ($p<0.06$). In another experiment, mice were intraperitoneally injected with the sense -47APP-PNA or saline control either daily for four days, twice daily for four days, or daily for 12 days. No change in brain Aβ(1-40) or Aβ(1-42) protein levels or mRNA levels was observed between the sense -47APP-PNA-treated mice and saline-treated control mice. In addition, the sense -47APP-PNA was not detected in the brain samples collected from the sense -47APP-PNA-treated mice. PNA oligomers (six total including the sense -47APP-PNA) were also not detected in brain samples collected from mice having had received a single intraperitoneal injection. The sense β-APP-PNA, however, was detected in brain samples collected from mice treated intraperitoneally with the sense β-APP-PNA daily for four days.

11. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the instant patent application or any patent issuing thereon.

Dated: 12/31/2001

Elliott Richelson

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